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Survival, Reproduction and Bioconcentration in Invertebrates and Fish Exposed to Hexachlorobenzene

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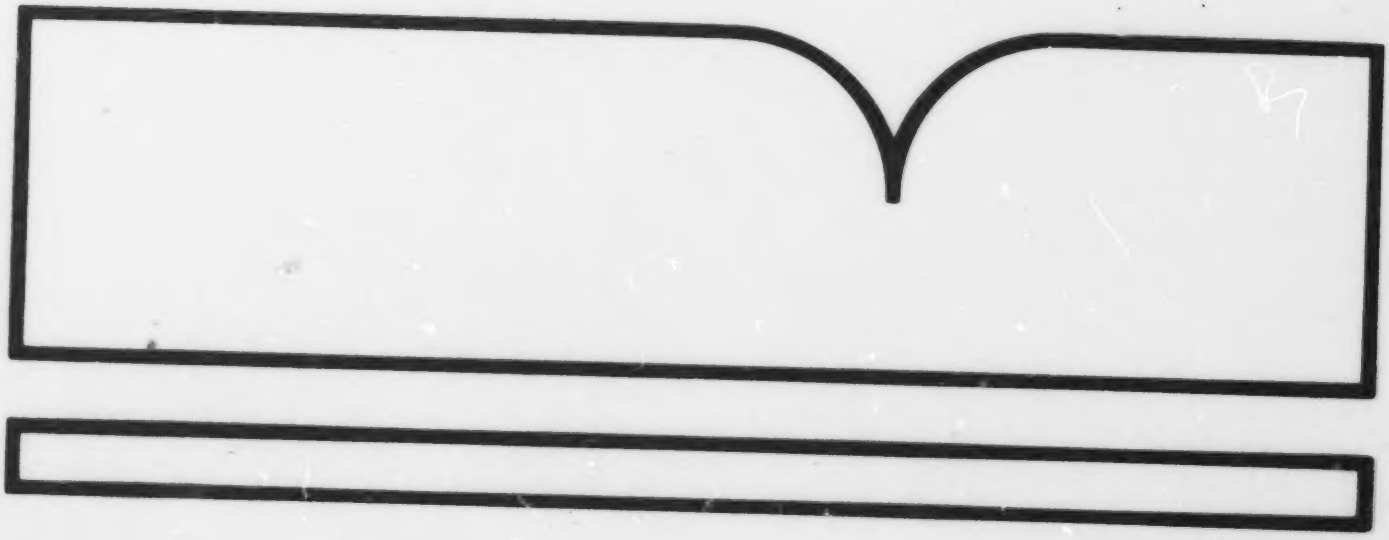

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The cladoceran *Daphnia magna*, the amphipods *Hyalella azteca* and *Gammarus lacustris*, the annelid worm *Lumbriculus variegatus* and the fathead minnow *Pimephales promelas* were exposed to hexachlorobenzene (HBC) in flow-through tests ranging from 2 to 68 d in duration to determine the effects of HCB on survival, tissue bioconcentration and *Hyalella* and *Lumbriculus* growth and reproduction.

No effects on survival, growth or reproduction were observed at concentrations of HCB up to saturation or at tissue HCB concentrations of up to 223 µg/g. Tissue HCB concentrations increased with increasing water concentrations, and tissue bioconcentration values were generally similar. Rapid uptake and change of tissue HCB concentration with change in water concentration occurred, with rapid depuration when the animals were no longer exposed to HCB in the water column, and tissue HCB concentrations were correlated closely with HCB water concentrations.

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SURVIVAL, REPRODUCTION AND BIOCONCENTRATION IN INVERTEBRATES AND FISH EXPOSED TO HEXACHLOROBENZENE

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Abstract—The cladoceran *Daphnia magna*, the amphipods *Hyalella azteca* and *Gammarus lacustris*, the annelid worm *Lumbriculus variegatus* and the fathead minnow *Pimephales promelas* were exposed to hexachlorobenzene (HCB) in flow-through tests ranging from 2 to 68 d in duration to determine the effects of HCB on survival, tissue bioconcentration and *Hyalella* and *Lumbriculus* growth and reproduction.

No effects on survival, growth or reproduction were observed at concentrations of HCB up to saturation (5.0 μg/L) or at tissue HCB concentrations of up to 223 μg/g. Tissue HCB concentrations increased with increasing water concentrations, and tissue bioconcentration values were generally similar. Rapid uptake and change of tissue HCB concentration with change in water concentration occurred, with rapid depuration when the animals were no longer exposed to HCB in the water column, and tissue HCB concentrations were correlated closely with HCB water concentrations.

Keywords—Hexachlorobenzene *Daphnia* *Hyalella* *Gammarus* *Lumbriculus* *Pimephales*

INTRODUCTION

Hexachlorobenzene (HCB) is a waste by-product of industrial chemical manufacturing and has been used as an agricultural fungicide. It is toxic at low levels after long-term exposure and has been found in mammals, birds, fish and soil [1]. There is little information in the literature about chronic exposure of freshwater invertebrates in flow-through test systems with measured HCB concentrations [1,2]. Because of the low solubility of HCB (approx. 5.0 μg/L) it may not be available to exposed aquatic animals in high enough concentrations to cause acute mortality [3]. Carlson and Kosian [4] concluded that HCB would not cause toxicity in aquatic organisms at or below water saturation concentrations. Chronic toxicity might occur in aquatic animals because of the gradual buildup of HCB in tissues, but the chemical is localized in lipids and may not be available even though high levels are found when animal tissues are analyzed for HCB. The high HCB concentrations that predators, including humans, are exposed to when eating contaminated organisms are a cause for concern because of the potential for carcinogenicity [5].

No freshwater invertebrate chronic tests or bioconcentration data are included in the U.S. Environmental Protection Agency (EPA) Water Quality Criteria document for chlorinated benzenes [2]. However, a fathead minnow (whole body) bioconcentration factor (BCF) for HCB of 22,000 is cited. Kosian et al. [6] reported fathead minnow BCFs of 48,000 to 52,000. Oliver [7] reported a BCF of 24,000 for oligochaete worms in laboratory studies using tissue/pore water. His data also indicated a BCF of 20,000 for rainbow trout.

The purpose of this study was to determine (a) if HCB would be toxic to the cladoceran crustacean *Daphnia magna*, the freshwater amphipod crustaceans *Hyalella azteca* and *Gammarus lacustris*, the annelid worm *Lumbriculus variegatus*, and the fathead minnow *Pimpehales promelas* under flow-through test conditions; (b) if HCB would have an effect on *Hyalella* and *Lumbriculus* growth and reproduction; (c) the rate of tissue HCB change with water HCB change; and (d) 30-d and longer-term tissue concentrations and bioconcentration values.

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MATERIALS AND METHODS

Test animals and procedures

A flow-through diluter system was constructed and used to prepare the exposure concentrations for these tests with the five freshwater test species. All animals were obtained from laboratory cultures [8]. Tissue samples were taken at the beginning and at the end of the tests combining survival, growth, reproduction and bioconcentration. For the longer uptake/depuration bioconcentration tests, animals were collected for tissue analysis twice a week. Water samples, 100 ml obtained with a pipette, were also collected when tissue samples were taken. Organisms removed during the bioconcentration exposures were blotted dry and placed in a 20-ml bottle with an aluminum foil-sealed cap and frozen for later (one–three weeks) HCB tissue analysis. Gut content was included in the analyses. Chapman [9] cautioned that worm gut content may cause erroneous results. Oliver [7] showed that oligochaete worm gut contents were 15% of dry weight and that fecal pellets contained the same chemical content as sediment, concluding that gut content contained a negligible amount of contaminant.

Daphnia *tests*

Daphnia magna of different ages, ranging from newly born to older adults, were used in each of several continuous-flow tests. Ten animals were placed in replicate 1,000-ml beakers with controls. Test duration ranged from 2 to 7 d and *Daphnia* were not fed during testing. No *Daphnia* were exposed in longer chronic tests for reproduction or HCB tissue analyses since the test water was too soft (25–35 mg/L as $CaCO_3$) to ensure that healthy *Daphnia* control populations could be maintained for extended periods.

Hyalella *tests*

Two 30-d survival, growth, reproduction and bioconcentration tests (Tests 1 and 2) and two 2-month bioconcentration tests (Tests 3 and 4) were conducted with *H. azteca*. In Test 1, 20 randomly selected *Hyalella* adults were placed in each test container (1,000-ml beaker) without additional substrate. In Test 2, 40 animals (20 young mated pairs to optimize reproduction) were placed in each beaker. Animals born during the tests were not used for tissue analysis. The animals in Tests 1 and 2 were fed twice a week, exposed for 30 d with controls, counted and weighed (blotted live weight), and frozen (one–three weeks) for later tissue analysis. Young were counted and removed on days 15 and 30 of the test. A mixed population of adults and juvenile *Hyalella* was placed in 20-liter aquaria, without additional substrate, for the longer bioconcentration studies (Tests 3 and 4); organisms (and water samples) were collected twice each week for tissue analysis. *Hyalella* were fed pelleted rabbit food, Cerophyl, newly hatched brine shrimp and pelleted frozen fish food. The HCB concentration in all foods used was measured at less than 0.4 μg/g.

Gammarus *tests*

One 28-d survival and bioconcentration test was conducted with *G. lacustris* in 1,000-ml beakers. Ten adults were placed in each beaker without additional substrate, with two beakers at each of five concentrations plus controls. They were fed pelleted rabbit food, frozen fish food and newly hatched brine shrimp, and analyzed in the same way as the *Hyalella*.

Worm tests

Four *Lumbriculus variegatus* tests were conducted. Long-term exposure of worms was conducted in a glass box (45 × 30 × 15 cm) containing sediment and receiving the combined flow-through effluent from all diluter concentrations. Worms of mixed ages were transferred directly from our laboratory cultures to the mud substrate in the box for the three bioconcentration exposures (Tests 1, 2 and 4). For the survival, growth, reproduction and bioconcentration test (Test 3), 10 mature worms were placed in a beaker containing a coarse quartz sand substrate at each of three continuous-flow HCB concentrations, plus controls, and exposed for 49 d; they were then counted, weighed and frozen for later tissue analysis. The worms were fed frozen pelleted fish food.

Fathead minnow tests

Young fathead minnows, 20 to 50 d old and 10 to 35 mm long, were used for the survival, growth and bioconcentration exposures; in any given test, fish were the same age and of similar size. Fathead minnows were fed newly hatched brine shrimp and pelleted frozen fish food that was determined to be free of detectable HCB contamination (<0.4 μg/g). In the survival, growth and bioconcentration test, 20 fish were placed in a beaker at each of the five concentrations plus controls, fed daily and exposed for 28 d. They were then counted, weighed, measured (standard length) and frozen for later HCB tissue analysis. The two 66-d bioconcentration tests

with fish were conducted using EPA [10] and American Society for Testing and Materials [11] procedures as general guidelines for measuring uptake and depuration tissue HCB concentrations, and for determining bioconcentration values. Seven grams of young fish was placed in the 20-liter aquarium for each test. Control fish from the same group were also placed in a 20-liter aquarium.

Test water

The water for these flow-through exposures was obtained from wells located 30 m from the Willamette River at Corvallis, Oregon. The water was vigorously aerated prior to use to stabilize gases and to eliminate supersaturated gas conditions caused by heating test water from ambient to 20°C. The water was relatively soft (hardness 25–35 mg/L as $CaCO_3$). The pH was 7.0 ± 0.3, and oxygen was near saturation at all times. The methods used for water chemical analysis were those described by the American Public Health Association [12].

HCB diluter facility and test conditions

A diluter consisting of a head box, mixing box and a six-chambered, partitioned dilution box was constructed and used to generate a saturated HCB solution with dilutions to be used for animal exposures (five concentrations of HCB plus controls). Glass columns (40–60 mm i.d. burettes) containing HCB-coated alundum boiling chips were mounted next to the mixing box on the diluter. The water in the mixing box (receiving water from the diluter head box and flowing into the first chamber of the dilution box) was recirculated with a small pump through the columns to provide increased contact time with HCB and water to bring the HCB water concentration to saturation (approx. 5.0 μg/L). The water flowed from the mixing box into the first chamber of the dilution box. The HCB solution was then diluted (0.5 dilution) with water from the head box flowing into the dilution box, from nominal 5.0 to 2.5, 1.2, 0.6 and 0.3 μg/L HCB, giving five concentrations plus controls. Glass lines delivered the HCB-containing water to test containers. Water was delivered directly from the diluter head box to the control test containers. Survival, growth, reproduction and 30-d bioconcentration tests were conducted in 1-liter beakers with a screened glass drain tube glued in a hole drilled in the side of the beaker.

Exposures of *Hyalella* and fathead minnows for the bioconcentration tests at 5.0 μg/L were conducted so that sufficient organisms could be held in the aquaria for the entire sampling period without exceeding the loading guidelines of 0.8 g tissue/L [13]. The flow rate to each test container was 70 ± 10 ml/min, giving a turnover rate of 4 volumes/h in the beakers and 6 volumes/d in the 20-liter aquaria. The diluter was monitored daily to ensure that proper flow rate was maintained. The temperature in the waterbath holding the beakers and aquaria was maintained at 20 ± 1°C, and the photoperiod was 16 h light:8 h dark. The diluter and waterbath were enclosed in a hood to vent HCB from the air surrounding the exposure system.

Analytical procedures

Water samples for HCB analysis, 100 ml from each concentration, were extracted with 25 ml ethyl acetate using magnetic stirring devices [14,15]. Sample extracts were dried using sodium sulfate and concentrated in Kuderna-Danish concentrators. No cleanup of sample extracts was necessary for this study. Samples were analyzed by electron-capture GC on a Hewlett-Packard Model 5730 gas chromatograph utilizing a 12.5-m chemically bonded cross-linked dimethyl silicone (OV-1) fused-silica capillary column. The chromatograph was equipped with a ^{63}Ni electron capture detector and operated in the split mode (about 75:1); the detection limit for HCB was about 50 pg. The detection limits for HCB in water were near 0.1 μg/L; the limits in food and tissue were near 0.4 and 0.1 μg/g, respectively.

Animals used for tissue HCB analysis were extracted with ethyl acetate using a Potter-Elvehjem tissue grinder [16]. Large *Gammarus* and fish were sometimes too resistant to grinding and so were frozen in liquid nitrogen and ground with a mortar and pestle before the extraction step. Sample extracts were dried with sodium sulfate and brought up to a suitable volume for analysis. Samples were analyzed by electron-capture GC, as above.

Controls (blanks), replicates and spikes were run during the course of the study. Ten percent of the samples were replicated and spiked. Recoveries of all spikes averaged 100 ± 8% and controls showed no interfering peaks.

Lipid determinations were made according to Association of Official Analytical Chemists Method 18.046 for fat in fish meal [17]. Samples of tissue were Soxhlet-extracted for 16 h with acetone, the acetone was evaporated, and the amount of lipid was determined gravimetrically. No acid hydrolysis or secondary extraction was done on the samples from this study. *H. azteca* contained 2.3 ±

0.6% lipid (mean of four samples). *G. lacustris* had 5.3 ± 0.4% lipid (mean of six samples). Percent worm lipid was determined to be 1.8 ± 0.2% of body weight. Percent fathead minnow lipid was determined to be 6.9 ± 0.4% of body weight. Lipid bioconcentration values were calculated from whole body bioconcentration values using the formula, lipid BCF = BCF × 100/lipid %.

RESULTS AND DISCUSSION

Daphnia *tests*

No significant *D. magna* mortality due to HCB concentrations up to water saturation (approx. 5.0 μg/L HCB) was observed with any age group or test duration. The lack of HCB toxicity was adequately demonstrated in these tests. The results are similar to those of Bobra et al. [18], who reported that the lack of toxicity and their inability to obtain an EC50 in *Daphnia* tests with HCB was predictable from the behavior of the chemical's congeners; they noted that the EC50 corresponds to a supersaturated, and therefore unattainable, concentration. Reproduction and bioconcentration tests could be conducted with this species to determine the possible impacts of longer exposure, and to determine if food chain bioaccumulation is a potential problem for avian or mammalian consumers. However, it appears from the literature and the series of tests conducted here that there probably is no toxicity of HCB to *Daphnia*.

Hyalella *tests*

No significant mortality of *Hyalella* adults due to HCB was seen during the two 30-d survival, growth and reproduction exposures (Table 1). Reproduction occurred at all HCB concentrations and in the controls, with no observed effects of HCB at concentrations of up to near water saturation (mean measured HCB concentration 4.5 μg/L). The mean number of young per beaker in Test 1 using mixed adults was 7.4; in Test 2, using mated pairs, it was 27. Adults and young appeared healthy and exhibited normal behavior at all HCB concentrations. *Hyalella* did very well in glass containers without additional substrate, and the undigestible food particles gave additional areas of cover. Tissue concentrations for the adult *Hyalella* were directly proportional to the water concentrations (Tables 1

Table 1. *Hyalella azteca* survival, growth, reproduction and hexachlorobenzene (HCB) tissue concentrations

Nominal HCB concn. (μg/L)	Beaker	Mean HCB concn. (μg/L ± SD)	Number alive at end of 30-d test	Mean adult weight (mg)	Number[a] of young/ beaker	Mean adult tissue HCB values (μg/g)	BCF	Lipid BCF (×1,000)
Test 1								
5.0	A	4.5 ± 1.8	9/10		2	172	38,220	1,662
	B		9/10	–	20			
2.5	A	3.3 ± 2.5	9/10		2	73	22,120	961
	B		9/10	–	12			
1.2	A	0.8 ± 0.4	8/10		0	60	75,000	3,261
	B		8/10	–	5			
0.6	A	0.7 ± 0.5	8/10		3	28	40,000	1,739
	B		10/10	–	1			
0.3	A	0.4 ± 0.3	8/10		13	14	35,000	1,521
	B		2/10[b]	–	2			
Control	A	<0.1	8/10		13	<3	–	–
	B		8/10	–	16			
Test 2								
5.0	A	3.8 ± 2.7	36/40	3.1	26	101	26,580	1,156
2.5	A	2.0 ± 1.1	39/40	3.5	17	49	24,500	1,065
1.2	A	0.7 ± 0.1	33/40	3.4	31	27	38,570	1,677
0.6	A	0.5 ± 0.1	33/40	3.4	38	14	28,000	1,217
0.3	A	0.3 ± 0.1	32/40	3.7	25	8.5	28,330	1,232
Control	A	<0.1	34/40	3.7	2[b]	<3.5	–	–

BCF, bioconcentration factor.
[a]Mated pairs were not used in Test 1. The numbers of young in Test 1 represent random pairing of some adults. Mated pairs were used in Test 2.
[b]Drain screen improperly fitted; lost most animals.

and 2) and the BCFs were generally similar at all concentrations. The close relationship between water concentration, which fluctuated from 4 to 6 μg/L in the diluter output, and tissue concentration can be seen in Figures 1 and 2, indicating how rapidly HCB in *Hyalella* came to equilibrium and the changes in response to environmental HCB concentrations. At day 49, when HCB delivery in the diluter was stopped, the tissue concentrations in the animals dropped rapidly. Figure 3 portrays the relationship and the BCF constancy throughout the water and tissue concentration range.

Gammarus *tests*

Significant mortality ($p = 0.05$) of *Gammarus* adults occurred at 5.0 μg/L, although it was not much greater than in other concentrations. The mortalities probably were not due to HCB toxicity, as the surviving animals' physical aspect and behavior appeared healthy and normal. Tissue

Table 2. *Hyalella azteca* and *Lumbriculus variegatus* tissue HCB concentrations and bioconcentration values after 28 and 42 d of exposure to hexachlorobenzene (HCB)

Test no.	Mean HCB concn. (μg/L ± SD)	Mean tissue HCB values (μg/g)		BCF (tissue/water)		Lipid BCF (×1,000)	
		28 d	42 d	28 d	42 d	28 d	42 d
Hyalella							
3	4.7 ± 0.9	63	62	13,400	13,190	583	573
4	3.2 ± 0.8	78	46	24,370	14,375	1,060	625
Lumbriculus							
1	1.4 ± 1.8	24[a]	–	17,140[a]	–	952	–
2	1.1 ± 0.2	24[b]	–	21,820[b]	–	1,212	–
4	0.8 ± 0.2	4[c]	–	5,000[c]	–	277	–
Controls	<0.06	<0.1	–	–	–	–	–

BCF, bioconcentration factor.
[a]Sediment exposed to water HCB concentration of 1.4 μg/L for 1 month prior to test start.
[b]Sediment exposed to water HCB concentration of 1.1 μg/L for 2 months prior to test start.
[c]Clean sediment; no prior exposure to HCB.

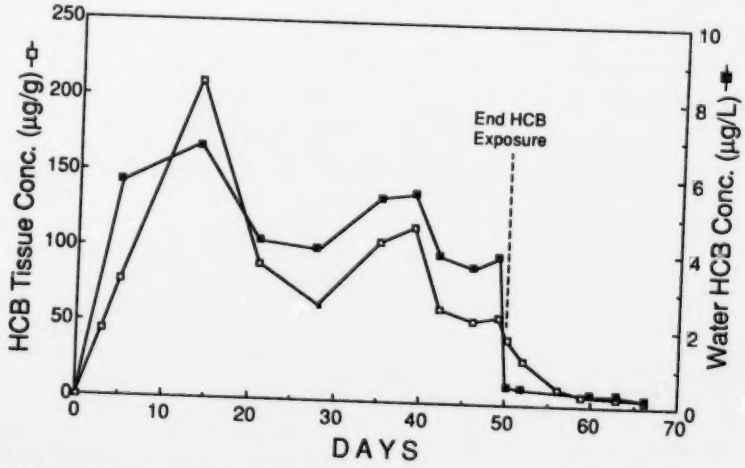

Fig. 1. Influence of water hexachlorobenzene (HCB) concentration (μg/L) on *Hyalella azteca* tissue HCB concentration (μg/g): Test 1.

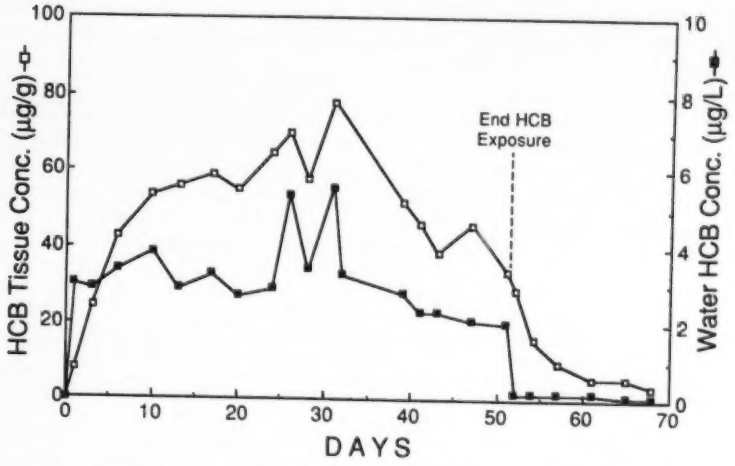

Fig. 2. Influence of water hexachlorobenzene (HCB) concentration (µg/L) on *Hyalella azteca* tissue HCB concentration (µg/g): Test 2.

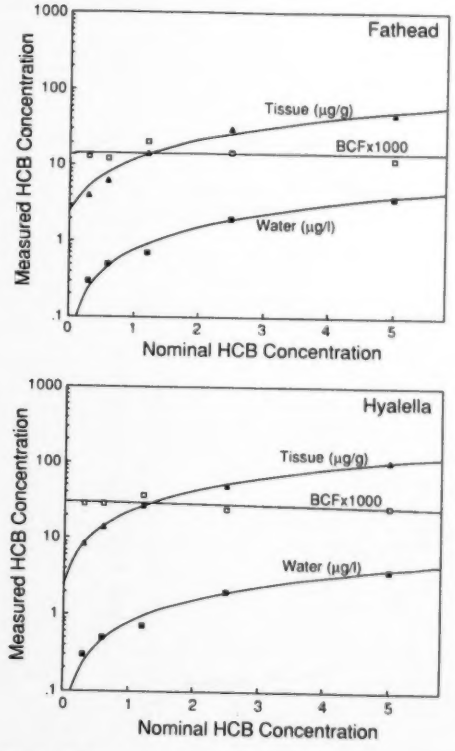

Fig. 3. Relationship of water concentration, tissue concentration and bioconcentration factor (BCF) for fathead minnows and *Hyalella azteca* exposed to hexachlorobenzene (HCB).

concentrations for the animals at each of the five concentrations are given in Table 3. The same relationship as for *Hyalella*—increased tissue concentration with increased water concentration—was apparent for *Gammarus*.

Worm tests

The oligochaete worms *L. variegatus* were exposed in one 28-d and two 2-month bioconcentration tests (Tests 1, 2 and 4). During these tests, no individual observations for mortality or growth were conducted; the worms were sampled for HCB tissue concentrations only. However, during the survival, growth and reproduction study (Test 3), in which 10 worms were placed in a beaker at each HCB concentration, worms were counted and weighed and analyzed for tissue HCB concentration. The influence of water HCB concentration (µg/L) on worm tissue HCB concentration can be seen in Figures 4 and 5. The tissue HCB concentration increased rapidly at the onset of exposure, paralleled the water concentration and dropped rapidly when HCB was no longer in the water.

No significant effects on survival, growth or asexual reproduction were observed (Table 4). Reproduction and growth occurred at all HCB concentrations in Test 3 and the worms thrived on the food and coarse quartz sand substrate in the 1,000-ml beakers. Worms do require an additional substrate to prevent them from constantly seeking shelter or forming balls of worms, which they do if placed in beakers without sand or sediment.

Table 3. *Gammarus lacustris* survival and hexachlorobenzene (HCB) tissue concentrations

Nominal HCB concn. (μg/L)	Beaker	Mean HCB concn. (μg/L ± SD)	Number alive at end of 28-d test	Average percent alive at end of 28-d test	Mean *Gammarus* tissue HCB values (μg/g)	BCF	Lipid BCF (×1,000)
5.0	A	3.3 ± 0.4	5/10	60	79	23,940	452
	B		7/10				
2.5	A	1.8 ± 0.3	6/10	80	53	29,440	555
	B		10/10				
1.2	A	1.0 ± 0.2	7/10	75	33	33,000	623
	B		8/10				
0.6	A	0.8 ± 0.2	8/10	80	15	18,750	354
	B		8/10				
0.3	A	0.4 ± 0.2	7/10	75	8	20,000	377
	B		8/10				
0.0	A	<0.06	9/10	90	<1.0	–	–

BCF, bioconcentration factor.

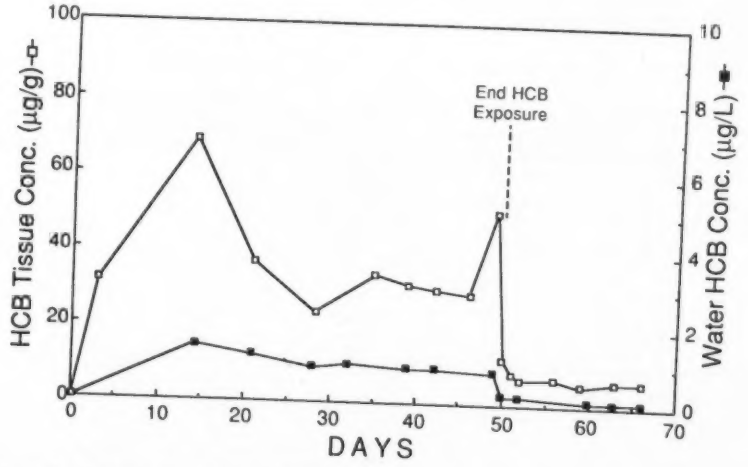

Fig. 4. Influence of water hexachlorobenzene (HCB) concentration (μg/L) on annelid worm (*Lumbriculus variegatus*) tissue HCB concentration (μg/g): Test 2.

From the original 10 animals placed in the beakers, there was an average of 152 worms per beaker after 7 weeks of exposure (Table 4). The worm HCB tissue concentration in Test 1, in which worms were exposed to 1.4 μg/L, was 24 μg/g after 28 d of exposure. Tissue HCB concentration in Test 2, in which worms were exposed to 1.1 μg/L, was also 24 μg/g after 28 d (Table 2). In Test 3, in which worms were exposed to three HCB concentrations (plus controls) for two months, the tissue concentrations after 28 d were 223, 203 and 50 μg/g (Table 4). The BCF for Test 1 after 28 d of exposure at 1.2 μg/L was 17,140; for Test 2, it was 21,820, and for Test 4, 5,000.

It is readily apparent that the unknown concentration of HCB in the sediment had a significant impact on the HCB tissue concentration in the worms and the BCF (Table 2). In Test 4 at 0.8 μg/L HCB water concentration, starting with clean sediment with no prior exposure to HCB, the tissue concentration after 30 d was only 4.0 μg/g, with a BCF (tissue/water) of 5,000. The sediment in Test 1 was exposed to an HCB water concentration of 1.4 μg/L for 1 month prior to and during

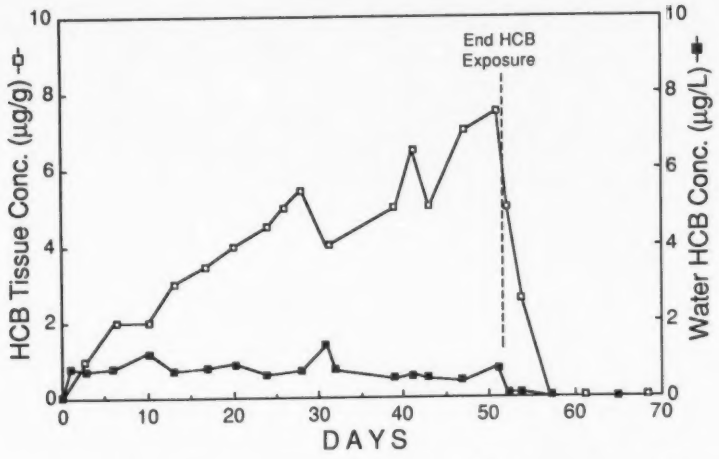

Fig. 5. Influence of water hexachlorobenzene (HCB) concentration (µg/L) on annelid worm (*Lumbriculus variegatus*) tissue HCB concentration (µg/g): Test 4.

Table 4. Worm (*Lumbriculus variegatus*) survival, growth, reproduction and hexachlorobenzene (HCB) tissue concentrations after 49 d of exposure to HCB (Test 3)

Nominal HCB concn. (µg/L)	Mean HCB concn. (µg/L ± SD)	Number[a] of worms alive at end of test	Mean individual worm weight (mg)	Mean worm tissue HCB values (µg/g)	BCF	Lipid BCF (×1,000)
5.0	4.7 ± 1.2	100	5.35	223	47,450	2,636
2.5	1.9 ± 0.4	222	4.57	203	106,840	5,936
1.2	1.0 ± 0.5	136	4.92	50	50,000	2,778
Control	<0.06	151	4.21	<0.1	–	–

BCF, bioconcentration factor.
[a]Started with 10 worms in each beaker; coarse quartz sand used as substrate.

the test, and the worms had a tissue concentration of 24 µg/g and a BCF of 17,140. The sediment in Test 2 was exposed to an HCB water concentration of 1.1 µg/L for 2 months prior to and during the test, and the worms had a tissue concentration of 24 µg/g and a BCF of 21,820. Worms exposed to water-only HCB concentrations in the beakers had much higher BCFs than did those exposed while living in sediment (Table 4).

Fathead minnows

No mortality of fathead minnows due to HCB toxicity was observed and the minnows grew rapidly at or below a concentration of 3.8 µg/L during the three tests conducted (Table 5). Close relationships between water and HCB concentration can be seen in Figures 6 and 7, indicating that HCB in the fish tissues came into equilibrium rather quickly with the water column HCB as the water concentration changed. The BCFs obtained for fish in Test 1 (Table 5) were very similar (Fig. 3) at different HCB concentrations, clearly indicating, at least in this test, that the BCFs were the same regardless of HCB water concentration. Tissue concentrations for the juvenile fathead minnows in Test 1, ranging from 4.0 to 46.5 µg/g HCB, were proportional to water concentrations. However, the bioconcentration values were similar, ranging from 12,240 to 21,140. From a straight line drawn through the five mean HCB concentra-

Table 5. Fathead minnow (*Pimephales promelas*) survival, growth and hexachlorobenzene (HCB) tissue concentrations after 28 d of exposure to HCB (Test 1)

Nominal HCB concn. (μg/L)	Mean HCB concn. (μg/L ± SD)	Number alive at end of 28-d test	Mean fathead HCB tissue values (μg/g)	Mean[a] standard length (mm ± SD)	Mean weight (mg ± SD)	BCF	Lipid BCF (×1,000)
5.0	3.8 ± 2.7	20/20	46.5	15.6 ± 2.5	68 ± 27	12,240	177
2.5	2.0 ± 1.1	20/20	30.5	16.5 ± 1.7	75 ± 25	15,250	221
1.2	0.7 ± 0.1	20/20	14.8	16.6 ± 2.0	72 ± 25	21,140	306
0.6	0.5 ± 0.1	20/20	6.3	17.1 ± 1.9	87 ± 27	12,600	182
0.3	0.3 ± 0.1	20/20	4.0	15.6 ± 2.5	77 ± 24	13,330	193
Control	<0.1	19/20	<1.0	16.5 ± 2.9	81 ± 33	–	–

BCF, bioconcentration factor.
[a]10–13 mm standard length at test start.

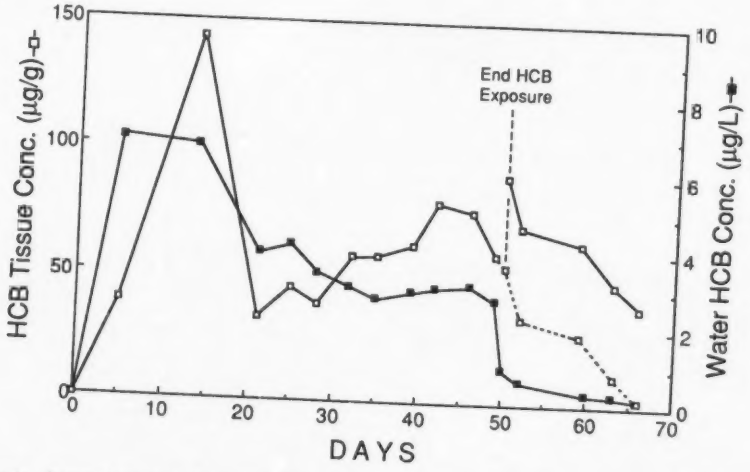

Fig. 6. Influence of water hexachlorobenzene (HCB) concentration (μg/L) on fathead minnow tissue HCB concentration (μg/g): Test 2.

tions, the mean measured value for the nominal concentration of 1.2 μg/L is near 1.0 μg/L, giving a BCF of 14,800, very close to the other values. This indicates that the mean measured value of 0.7 μg/L probably does not truly reflect the actual mean HCB value during the test period. Tissue concentrations in Tests 2 and 3 were 39 and 62 μg/g, with BCFs of from 11,470 to 20,660. Articles by Veith et al. [19], Ahmad et al. [2] and Kosian et al. [6] present BCFs for fathead minnows of 16,000 to 18,500, 23,391 and 48,000 to 52,000, respectively, generally similar to the results of this study.

CONCLUSIONS

Absence of HCB toxicity to the five test species was confirmed in these studies and no effects on growth or reproduction were observed. HCB is a good chemical to use for bioconcentration studies because of its lack of toxicity, low biodegradability and high bioconcentration. A period of 28 d is adequate to show bioconcentration equilibration. HCB tissue concentrations change rapidly as water concentrations change. Bioconcentration values for HCB are independent of water concentration. *Hyalella* and *Lumbriculus* reproduction tests are new

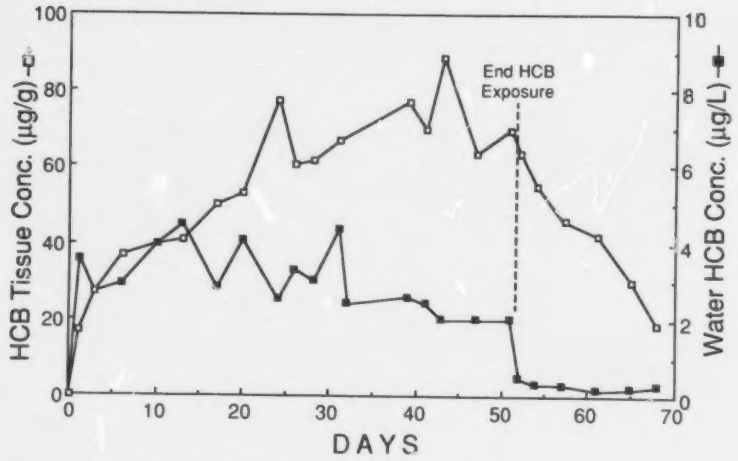

Fig. 7. Influence of water hexachlorobenzene (HCB) concentration (µg/L) on fathead minnow tissue HCB concentration (µg/g): Test 3.

and will be used in future tests to determine chronic effects of toxicants or unknown mixtures (e.g., effluents, sediments, Superfund site materials).

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REFERENCES

1. **Courtney, K.D.** 1979. Hexachlorobenzene (HCB): A review. *Environ. Res.* **20**:225–266.
2. **Ahmad, N., D. Benoit, L. Brooke, D. Call, A. Carlson, D. DeFoe, J. Huot, A. Moriarity, J. Richter, P. Shubat, G. Veith** and **C. Wallbridge.** 1984. Aquatic toxicity tests to characterize the hazard of volatile organic chemicals in water: A toxicity data summary—Parts I and II. EPA-600/3-84-009. U.S. Environmental Protection Agency, Duluth, MN.
3. **U.S. Environmental Protection Agency.** 1980. Ambient water quality criteria for chlorinated benzenes. EPA 440/5-80-028. Washington, DC.
4. **Carlson, A.R.** and **P.A. Kosian.** 1987. Toxicity of chlorinated benzenes to fathead minnows (*Pimephales promelas*). *Arch. Environ. Toxicol.* **16**:129–135.
5. **Sax, N.I.** 1984. Hexachlorobenzene. *Dangerous Properties of Industrial Materials*, 6th ed. Van Nostrand Reinhold, New York, NY.
6. **Kosian, P., A. Lemke, K. Studders** and **G. Veith.** 1980. The precision of the ASTM bioconcentration test. EPA-600/3-81-022. U.S. Environmental Protection Agency, Duluth, MN.
7. **Oliver, B.G.** 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. *Environ. Sci. Technol.* **21**: 785–790.
8. **Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema** and **D.F. Krawczyk.** 1984. Biological methods for determining the toxicity of contaminated freshwater sediments to invertebrates. *Environ. Toxicol. Chem.* **3**:617–630.
9. **Chapman, P.M.** 1985. Effects of gut sediment contents on measurements of metal levels in benthic invertebrates—A cautionary note. *Bull. Environ. Contam. Toxicol.* **35**:345–347.
10. **U.S. Environmental Protection Agency.** 1982. Fish bioconcentration test EG-10. Environmental Effects Test Guidelines, Part One. EPA 550/6-82-002. Office of Pesticides and Toxic Substances, Washington, DC.
11. **American Society for Testing and Materials.** 1984. Proposed new standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. Draft No. 8, E-47.01. Philadelphia, PA.
12. **American Public Health Association, American Water Works Association** and **Water Pollution Control Federation.** 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. American Public Health Association, Washington, DC.
13. **American Society for Testing and Materials.** 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-80. Philadelphia, PA.
14. **Ripley, B.D., J.W. Wilkinson** and **A.S.Y. Chau.** 1974. Multiresidue analysis of fourteen organophosphorus pesticides in natural waters. *J. Assoc. Off. Anal. Chem.* **57**:1033–1042.
15. **Muir, D.C.** and **B.E. Baker.** 1976. Detection of tri-

azine herbicides and their degradation products in tile-drain water from fields under intensive corn (maize) production. *J. Agric. Food Chem.* **2**:122–125.

16. **U.S. Environmental Protection Agency.** 1980. Manual of analytical methods for the analysis of pesticides in humans and environmental samples. EPA-600/8-80-038. Washington, DC.
17. **Williams, S.**, ed. 1984. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed. Association of Official Analytical Chemists, Arlington, VA.
18. **Bobra, A., W.Y. Shiu** and **D. MacKay.** 1985. Quantitative structure–activity relationships for the acute toxicity of chlorobenzenes to *Daphnia magna*. *Environ. Toxicol. Chem.* **4**:297–305.
19. **Veith, G.D., D.L. DeFoe** and **B.V. Bergstedt.** 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. *J. Fish. Res. Board Can.* **36**: 1040–1048.

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